I. CHEMISTRY OF SPHINGOSINE
II. METABOLISM OF FATTY ACIDS

BY

FRANCIS JAMES GLICK

B.A., De Pauw University, 1939

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN
CHEMISTRY IN THE GRADUATE SCHOOL
OF THE UNIVERSITY OF ILLINOIS, 1943

URBANA, ILLINOIS

1943

I. CHEMISTRY OF SPHINGOSINE
II. METABOLISM OF FATTY ACIDS

BY
FRANCIS JAMES GLICK
B.A., De Pauw University, 1939

AN ABSTRACT OF A THESIS
SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN
CHEMISTRY IN THE GRADUATE SCHOOL
OF THE UNIVERSITY OF ILLINOIS, 1943

URBANA, ILLINOIS

1943

ACKNOWLEDGMENT

The author wishes to express appreciation to Dr. H. E. Carter for his advice, suggestions, and interest throughout the course of these investigations.

PART I

CHEMISTRY OF SPHINGOSINE

Introduction

Cerebrosides and sphingomyelin differ from the other lipids in nervous tissue in that they are derivatives of the complex base-sphingosine. The object of this investigation was to establish the correct structure of sphingosine as the first step in a study of the metabolism and function of its derivatives.

Experimental and Discussion

Refrigerated bovine spinal cords were macerated and extracted successively with acetone, ether, and hot alcohol. A mixture of cerebrosides and sphingomyelin—referred to as sphingolipids[1]—precipitated from the cooled alcoholic extracts. Crude cerebrosides were obtained by extraction of sphingomyelin from the sphingolipids with acetic acid.

The cerebrosides were hydrolyzed by the method of Klenk and Diebold[2]; sphingosine was isolated in amorphous form. Levene and West[3] have suggested that sphingosine is 1-amino-2,3-dihydroxyheptadecen-4. Klenk and Diebold[2] demonstrated sphingosine to have a C_{18} chain with a double bond between carbon atoms 4 and 5, and an animo and two hydroxyl groups on the three terminal carbon atoms. Without conclusive evidence they proposed that sphingosine is 1,2-dihydroxy-3-aminooctadecene-4.

At the beginning of this investigation, excellent methods were devised for the preparation of *N*-acyl and triacyl derivatives of sphingosine and of dihydrosphingosine. In general, sphingosine and its derivatives could be readily reduced catalytically to

dihydrosphingosine and its corresponding derivatives. Triacetylsphingosine however absorbed approximately 150 per cent of the amount of hydrogen theoretically required to saturate the double bond. The solvent at the end of the hydrogenation of 3.5 m*M* of triacetylsphingosine contained 2 m*M* of free (acetic) acid. An *O*-acetyl group apparently is located on carbon atom 3 of triacetylsphingosine and a part of the molecules undergoes hydrogenolysis before the double bond becomes saturated. Examples of hydrogenolysis of allylic esters have been reported by Bergström and Wintersteiner[4] and others. The above observation was the first evidence which contradicted the structure of sphingosine proposed by Klenk and Diebold.

Conclusive proof that the correct structure of sphingosine is

$$CH_3(CH_2)_{12}CH{=}CH\text{-}\underset{OH}{CH}\text{-}\underset{NH_2}{CH}\text{-}\underset{OH}{CH_2}$$

was obtained in the following manner. *N*-Benzoyldihydrosphinosine was treated with periodic acid under a variety of conditions. There was no reaction; the *N*-benzoyldihydrosphingosine was recovered and converted in 90 per cent yield to tribenzoyldihydrosphingosine for purposes of identification. But periodic acid is a specific and strong oxidizing agent for 1,2-glycols. Nicolet and Shinn[5] have shown that serine reacts readily with periodic acid where as *N*-acetylserine is not attacked by this reagent. The inertness of *N*-benzoyldihydrosphingosine toward periodic acids is strong evidence against the structures proposed by Levene and Klenk as each of these contains a 1,2-glycol group.

The above proof of the structure of *N*-benzoyldihydrosphingosine rests on the assumption that the three functional groups in dihydrosphingosine are on adjacent carbon atoms. To establish this assumption as valid, dihydrosphingosine was oxidized with periodic acid; equimolecular amounts of palmitaldehyde, formaldehyde, formic acid, and ammonia were isolated. The fact that a cyclic benzylidene derivative of *N*-benzoyldihydrosphingosine can be made[6] is additional evidence that the functional groups in dihydrosphingosine are on adjacent carbon atoms.

By these experiments sphingosine has been demonstrated to be 1,3-dihydroxy-2-aminooceadecene-4.

Bibliography

[1] Carter, H. E., Proc., 105th Meeting, American Chemical Society, Detroit, Mich., April 12-16, 1943.

[2] Klenk, E., and Diebold, W., Z. physiol. Chem., **193**, 25 (1931).

[3] Levene, P. A., and West, C. J., J. Biol. Chem., **16**, 549 (1913-14).

[4] Bergstrom, S., and Wintersteiner, O., J. Biol. Chem., **143**, 503 (1942).

[5] Nicolet, B. H., and Shinn, L. A., J. Biol. Chem., **139**, 687 (1941); J. Am. Chem. Soc., **61**, 1615 (1939).

[6] Carter, H. E., Glick, F. J., Norris, W. P., and Phillips, G. E., J. Biol. Chem., **142**, 449 (1942).

PART II.

METABOLISM OF FATTY ACIDS

Introduction

This study of the oxidation of branched-chain fatty acids by slices of guinea pig liver was undertaken primarily to gain some additional knowledge of the intermediate metabolism of fatty acids and of amino acids, especially valine, leucine, and isoleucine.

The following types of acids were studied:

$$CH_3(CH_2)\underset{\displaystyle (CH_2)_y \atop \displaystyle CH_3}{CH}\text{—}COOH \qquad CH_3(CH_2)\underset{\displaystyle CH_3}{CH}\text{—}CH_2COOH$$

The rate of oxygen consumption and production of acetone bodies were correlated with variations in chain length and position of the alkyl substituent. In drawing conclusions from the results obtained, it is to be borne in mind that only exploratory experiments on the problem have been performed.

Experimental and Discussion

The Warburg apparatus was calibrated by a gravimetric method described by Dixon[1]. The tissue slice technique of Quastel and Wheatley[2] was used for determining the influence of the

various fatty acids on respiratory rates. The aniline citrate method of Edson[3] was used for the acetoacetic acid analyses. Total acetone bodies and preformed acetone plus acetoacetic acid were estimated by the iodometric method of Barnes and Wick[4].

α-Alkyl-substituted fatty acids increased the normal respiratory rate of liver tissue as follows (in per cent): Isobutric acid, + 92; α-methylbutric acid, + 80; α-ethylbutric, + 20; α-propylbutric acid, —3. Alkyl substituents larger than methyl in the α-position inhibit oxidation markedly. None of these acids was catabolized to acetone bodies.

β-Methylbutyric (isovaleric) acid was quite readily oxidized by liver slices producing acetone and acetoacetic acid. β-Methylvaleric acid also yielded acetone and acetoacetic acid but less rapidly than isovaleric acid. In fact, the yield of catabolites was so small that a macro tissue slice method had to be developed to prove definitely that β-methylvaleric acid is a precursor of acetone bodies. The mechanism by which acetone bodies can be formed from β-substituted acids and the question of whether the acetone bodies so formed consist mainly of acetone are two problems which are as yet undecided.

Bibliography

[1] Dixon, M., Manometric Methods, The University Press, Cambridge, 1934, page 9.

[2] Quastel, J. H., and Wheatley, A., Biochem. J., **27**, 1753 (1935).

[3] Edson, N. L., Biochem. J., **29**, 2082 (1935).

[4] Barnes, R. H., and Wick, A. N., J. Biol. Chem., **131**, 413 (1939).

VITA

The author was born near Columbus, Indiana, on October 9, 1916. He was graduated from Columbus High School in June, 1935, and entered De Pauw University as a Rector Scholar the following September. In June, 1939, he was granted the degree of Bachelor of Arts with honors by De Pauw University. He entered the Graduate School of the University of Illinois in June, 1939, serving as a half-time assistant in biochemistry during his first two years of graduate study and holding a university fellowship during his third year. During the summer of 1942 he worked in the Research and Development Department of the National Aniline Division of the Allied Dye and Chemical Corporation. Since September, 1942, he has been employed as chemist on a government research project at the University of Illinois College of Medicine.

Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41549818_0177